

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

134

From Page No. \_\_\_\_\_

48  $\mu$ l of premix aliquoted to pre labeled appendants 4/1

1	1/200	1	} post heat kill
2		2	
3	1/500	1	
4		2	

5	1/500	1	} pre heat kill
6		2	
7	1/500	1	
8		2	

incubate @ 74°C in a heated water block - for 10 minutes  
quench w/ 10  $\mu$ l of 5M EDTA - spot 30  $\mu$ l on GPC

Wash 1x 10% TCA 1x Di 5'  
3x 5% TCA  
2x 5% H<sub>2</sub>O

dry + count w/ Econofluor

USER: 2 ID: 32P  
SAMPLE REPEAT:  
H#: 1 AQC: N QCF  
CHANNEL 1-LL:  
DATA CALC: CPM  
HALF LIFE (DAYS):

SAM	CPM1
1/200	8460.00
2	21680.00
1/500	8296.00
4	7486.00
1/200	16274.00
6	28614.00
1/500	8412.00
8	17912.00
9	2794.00
10 S.A.	91294.00

} post heat kill	11.9
	17.88
	29.7
	28.4
} pre heat kill	30.1
	29.54
	29.7

S.A. 57 cpm/pmol

mf  
6/20/95

To Page 1

Witnessed & Understood by me,

Date

Inv. nted by

Date

May Jones

6/20/95

R c rd d by

Elizabeth

06/19/95

'ag N .\_\_

12Y. SDS PAGE - 3-5' exo minus the



5/19/10

12/6/20/55

1	2	3	4	5	6	7	8	9	10
1 $\mu$	3 $\mu$	5 $\mu$	5 $\mu$	10 $\mu$	20 $\mu$		1 M		
pre heat kill			post heat kill						

u heat - @ 20  $\mu\text{g}/\mu\text{l}$  - spun down sup loaded on gel  
st heat @ .36  $\mu\text{g}/\mu\text{l}$

To Page No.\_\_\_\_\_

ssed & Understood by me ,

May Long

**Date**

6/20/95

**Invented by**

Record d by

3.4 gm

Date \_\_\_\_\_

06/16/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

FY1 - S. Sgm crack

From Page No. \_\_\_\_\_

Purpose: to screen FY1 - mutant Trn - one base point mutation phenylalanine to Tyrosine 1 for thermostable polymerase activity.

S. Sgams cells - resuspend in 10 mL of crack buffer - p. 132 ~ 15

Divide in two 7.5 mL samples in 15 mL conical  
Sonicate w/ microtip @ max output - 5  
6 x 20 sec bursts

Before - A<sub>595</sub> .750  
After - A<sub>595</sub> .320 ~ 57% crack

Sonicate again - 3 x 20 sec bursts

Before A<sub>595</sub> .750 ~ 73% crack  
After A<sub>595</sub> .200

Divide Save 400 mL - pre crack material  
Aliquot remainder of crack into 2 mL eppendorf  
incubate @ 87°C 11 minutes -  
Spin in microfuge 20 minutes @ 14,000 <sup>3</sup>

Decant and save supernatant → assay for  
thermostable polymerase activity -

To 500  $\mu$ L of TRN premix add 1.1  $\mu$ L of <sup>p32</sup> dCTP

48  $\mu$ L of premix / rxn - 1.2  $\mu$ L of  
diluted samples added incubate @  
74°C in a heated water block -  
for 10'. Quench rxn w/ 10  $\mu$ L of

1	1/200	1
2		2
3	1/400	1
4		2
5	1/200	1
6		2
7	1/400	1
8		2

5M EDTA - spot 30  $\mu$ L of GFI C  
Wash 1x w/ 10% TCA 1x PC @ 5  
3x w/ 5% TCA @ 3'  
1x w/ EDTA

dry + count -

To Page No.

Witnessed &amp; Understood by m ,

Date

Initiated by

Date

Recorded by

May Longo

6/20/95

E. Flynn

6/16/95

Page N. \_\_\_\_\_

USER: 2 ID: 32P  
 SAMPLE REPEAT:  
 H#: 1 ACC: N QCF  
 CHANNEL 1-LL:  
 DATA CALC: CPM  
 HALF LIFE (DAYS)

Note: Background very high!

06/14

Debris note: Did not induce for very long-

SAM CPM1

✓ 1 5722.00  
 2 7676.00  
 3 2608.00  
 4 4686.00  
 5 10454.00  
 6 19114.00  
 7 5850.00  
 8 11594.00  
 9 2976.00  
 10 90102.00  
 11 88418.00

Post

5.8

5.4 U/ul

Heat Kill

5.0

loss of 70%?

Pre Heat Kill

15.9 U/ul

17.1 U/ul

17.0 U/ul

18.3

my 6/20/95

Appears to have (very high background) +  
 lost activity after heat kill  
 \* maybe mostly lost D1 activity in pre heat kill?

Bradford's

Slope - .0555 0.0/ug

ul

0.0.

19/ul

Heat Kill Tyl - 40

.474

\* too ↑

.2 ug/ul

20

.333

.289 ug/ul

Pre Heat Kill Tyl - .

.259

.439

23.3 ug/ul

19.8

Post Heat Kill

.303

.136 ug/ul

3-5' zero mat

Run 12x SDS PAGE - see p 138

1-2-3-4

↓

To Page No. \_\_\_\_\_

Used &amp; Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Recorded by

06/16/95

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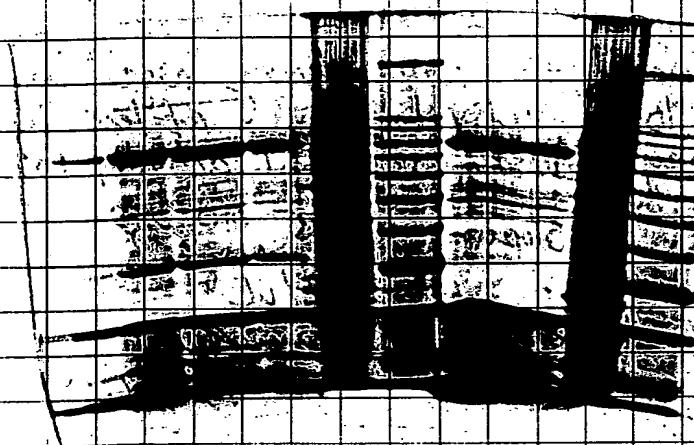
TITLE FYI + 3'-5' exo mutant SDS PAGE

From Page No. \_\_\_\_\_

06/11

12% SDS PAGE

1 2 3 4 5 6 7 8 9 10

1 6 $\mu$ g 4 $\mu$ g 2 $\mu$ g 60 $\mu$ g M 4 $\mu$ g 2 $\mu$ g 60 $\mu$ g MFYI  
post heat  
killFYI  
pre heat  
kill3'-5' exo M  
post heat  
killpre heat  
kill 3'-5' exo-

100kd

50kd

DVT 6/20/95

GTF  
C

pre heat kill - spun down sup loaded on gel

To Page 1

Witnessed &amp; Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Record d by

S. Flynn

06/16/95